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25 October 2000

Dockets Management Branch
Food and Drug Administration
5630 Fishers Lane, Room 1061 0985 '00 OCT 31 P12:50
Rockville, MD 20852

Re: Docket No. 00N-1409 Iontophoresis Device

To Whom It May Concern:

These comments are submitted in response to the 22 August 2000 proposed rule to revise the classification and identification for iontophoresis device as this appears in 21 C.F.R. § 890.5525. The proposal, if implemented, would limit the use of iontophoresis to drugs for which ". . . the labeling of the drug intended for use with the device bears adequate directions for the device's use with that drug."

The undersigned expresses objection to the proposed revision, because iontophoretic devices, like syringes, represent a method of drug delivery that is determined by or on the order of a licensed practitioner. Some drugs that are in solution may be safely and effectively administered by iontophoretic devices in accordance with the order of a licensed practitioner. Whether such drugs are compounded on the prescription of a licensed practitioner or otherwise available for injection but not labeled for iontophoretic use, the licensed practitioner is entitled to exercise a choice. This choice should not be limited by the absence of information on a drug label. The licensed practitioner must have the flexibility to use his/her skills consistent with his/her knowledge and experience.

With regard to the use of iontophoresis in cystic fibrosis ("CF") diagnosis, both the current and proposed regulations as written are confusing. The qualifying phrase having to do with drug labeling could be interpreted to apply to all uses including the diagnosis of cystic fibrosis, or it could be interpreted as applying only to "other uses." If the Food and Drug Administration ("FDA") intends to construe the proposed regulation to exclude the diagnosis of cystic fibrosis from the drug labeling requirement, then the following comments are not applicable. On the other hand, since the regulation could be construed to require adequate drug labeling for the diagnosis of cystic fibrosis, the undersigned offers the following comments for the record.

It is the belief of the undersigned that no drug in commercial distribution prior to 28 May 1976 or at present is explicitly labeled for cystic fibrosis diagnosis through delivery by iontophoresis. Yet, for example, pilocarpine salts have been used for this purpose continuously since 1959 when Gibson and Cooke first reported the use of pilocarpine iontophoresis as a means of stimulating sweat. A copy of their frequently referenced article, published in the journal *Pediatrics,* is enclosed. The two pediatricians had discovered a safe sweat induction method

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that would henceforth and forever replace induced hyperthermia as the means of stimulating the patient to produce sweat during a "sweat test." The complete procedure involves (1) sweat induction, (2) sweat collection, and (3) laboratory analysis of the collected sweat specimen to confirm or exclude a clinical diagnosis of CF.

The application of pilocarpine iontophoresis for the induction phase of the sweat test has been known to the Food and Drug Administration ("FDA") for at least twenty (20) years. For example, in the proposed rule in the Federal Register ("F.R.") of 28 August 1979, pages 50520-50522, several articles are cited, at least three of which contain specific reference to this method of sweat induction in sweat testing. Copies of the articles by Gibson, et al., Kopito, et al., and Tocci, et al. are included herewith for ease of reference. When the FDA, with the assistance of an expert advisory committee, proposed classification of the iontophoresis device, it was common knowledge that various substances were used to induce sweating. These substances were not to be used for any therapeutic purpose and the stimulation of sweat glands through use of these substances did not in and of itself produce the diagnosis.

Compliance with the classification requirements of the Medical Device Amendments of 1976 generated a final rule that has been in effect for nearly twenty (20) years. An advisory committee of experts met during open sessions in accordance with requirements of the Federal Food, Drug, and Cosmetic Act ("Act") and regulations appearing in 21 C.F.R. Part 860. Notice of these meetings was provided in the F.R. prior to the meeting of the committee and the agendas were announced in advance. The public was invited to attend and express their views. Moreover, the committee included one member to represent the views of consumers. The committee deliberated in public and prepared a report consistent with requirements of the Act and regulations. The FDA, in consideration of the advice provided by the committee, published a proposal in the F.R. on 28 August 1979. The preamble to this regulation provided a thorough discussion and identified reference documents including the transcript of the public hearing. All comments received from the public were evaluated over a several year period and on 23 November 1983 a final rule was published in the F.R.

There was no objection to the identification as this appears in 21 C.F.R. § 890.5525; and, to the best knowledge of the undersigned there has never been an incident to suggest that use of soluble salts and drugs such as pilocarpine have caused injury. The lack of any such report in the professional literature or elsewhere is compelling evidence of the safety of pilocarpine iontophoresis in view of the enormous number of sweat tests that have been performed in hospitals, clinics, and CF referral centers. Based on the experience of the undersigned, it is estimated that the total spectrum of sweat testing activity using various commercial and privately produced iontophoresis equipment during the past 40 years has included the performance of at least five (5) million individual pilocarpine iontophoresis procedures. As long as the iontophoretic device manufacturer does not make claims about use of drugs other than pilocarpine to aid in cystic fibrosis diagnosis, there should be no reason to impose greater strictures on sweat testing or on the means used to facilitate sweat testing.

The undersigned estimates that approximately twenty (20) thousand sweat tests are performed each month in hospitals, clinics, and CF centers worldwide; but in spite of the large number of tests, the quantity of drug consumed is relatively insignificant. It is highly unlikely that the pharmaceutical companies who manufacture soluble salts or drugs used for this purpose would be willing to obtain approval through a new drug application for iontophoretic delivery of their drugs. If the proposed rule were to be implemented as published and construed to require adequate drug labeling for CF diagnosis, the legality of these procedures in the United States would be brought into question and the time-honored laboratory diagnosis of CF could come to an abrupt halt. The consequences to the public health both here and abroad would be devastating if there was an unfortunate shutdown of this critically needed diagnostic procedure with a long history of safe and effective use.

At the very least, the FDA should amend the definition of iontophoresis device to clarify that the drug labeling requirement does not apply to the diagnosis of cystic fibrosis. Better still, the FDA would be wise to confirm that soluble salts or drugs used at the direction of licensed practitioners do not require adequate directions for use in the labeling of the drug. As an alternative, the FDA should consider acceptance in the labeling for a device the identification of soluble salts and drugs that can be used to induce sweating for cystic fibrosis diagnosis. This acknowledgment can be accomplished through submission of premarket notifications and jointly reviewed by the Centers for Devices and Radiological Health and Drug Evaluation and Research through the existing intra-agency agreement.

For the reasons cited above, including the extensive administrative record established prior to 23 November 1983 and the safe and effective use of iontophoretic devices to the present, the proposed rule should either be amended or abandoned. The FDA should consider reclassification of iontophoretic devices into either Class I or II as drug delivery systems comparable to syringes, pumps, and other comparable drug delivery devices.

Sincerely,

WESCOR, INC

Wayne K. Barlow
President/CEO

WKB:su

Enclosures

CLIN. CHEM. 22/11, 1841–1844 (1976)

Ref. on 1976 (20)

Laboratory Confirmation of the Diagnosis of Cystic Fibrosis

Paul M. Tocci and Robert M. McKey, Jr.

The recent commercial introduction of a method for detecting albumin in meconium makes screening for cystic fibrosis feasible for many hospitals. If the test is adopted, confirmatory tests should be available. Quantitative analyses of sweat for sodium by flame photometry and for chloride by silver titration and ion-selective electrodes are now used as confirmatory tests. We compare results of these confirmatory methods applied to persons with cystic fibrosis, respiratory disorders, or digestive disorders, and to control subjects.

Additional Keyphrases: *respiratory disorders • digestive disorders • Na^+ and Cl^- determination in sweat • albumin in meconium • pediatric chemistry • screening • inherited disorders • normal values • Na^+, Cl^-, K^+ correlations*

Cystic fibrosis (CF), an autosomal recessive inherited disease, occurs with a frequency of about 1:1600 live births (*1*). The diagnosis is usually made after symptoms appear. Some evidence indicates that life expectancy may be prolonged if intensive therapy is begun early in life, before pulmonary disease has developed (*2, 3*). Accordingly, a screening test[1] for the content of albumin in the meconium of newborns is under investigation (*4*). The albumin content of meconium is usually greater in 80–85% of children with CF because of pancreatic insufficiency than in control children (*5–7*). This test is simple to perform, relatively inexpensive, and sufficiently reliable to detect most infants with the disorder (*2*), but has the disadvantage that high false-positive rates have been reported as compared to the traditional sweat test (*4*).[2] Because the test does detect most cases of the disorder, it is likely that it will be adopted by some obstetric and neonatal services. This situation will cause needless anxiety for some parents if the laboratory is unprepared to confirm the diagnosis quickly. A presumptive diagnosis of CF produces severe emotional, social, and financial stress on a family. Therefore it is extremely important that the diagnosis not be stated until the patient, the family history, and the laboratory evidence have been carefully appraised.

University of Miami School of Medicine, Department of Pediatrics, P.O. Box 520006, Biscayne Annex, Miami, Fla. 33152.

[1] Boehringer No. 10038; Boehringer-Mannheim Corp., 219 E 44 St., New York, N.Y. 10017.

[2] Bruns, W. T., BMC-test on meconium in newborns: Test for cystic fibrosis. Cystic Fibrosis Club Meeting. National Cystic Fibrosis Research Foundation (August 1975). Abstract.

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In our Cystic Fibrosis Center a positive sweat test and two of three criteria are required for the diagnosis of CF: (*a*) the presence of the characteristic lung disease, (*b*) the presence of pancreatic insufficiency, and (*c*) a positive family history. In many cases the laboratory evidence becomes critical to the final decision. Therefore all positive screening tests must be confirmed as soon as possible to avoid emotional stress to a family.

The recognition of markedly increased sodium and chloride concentrations in sweat of patients with CF led to the use of the sweat test as a definitive diagnostic procedure (*8–10*). The Gibson–Cooke quantitative pilocarpine iontophoresis test is now the most reliable single diagnostic test for CF (*11–14*). In it, sweating is stimulated and sweat collected and analyzed for sodium or chloride, or both. The sweat-gland defect in CF is probably not acquired and it can be reliably demonstrated in 98% of such patients after three months of age.

Almost all hospital laboratories are equipped to do well-controlled analysis for sodium in serum or urine specimens by flame photometry and for chloride by silver titration. Technically, determination of these ions in sweat is very similar once the specimen has been collected. In the pilocarpine iontophoresis sweat test, difficulties occur in sample collection and handling. Evaporation is fast and can lead to false-positive tests by the artificial increase in sodium and chloride concentration. Any laboratory can perform the Gibson–Cooke sweat test accurately if there has been proper attention to detail in collecting and preparing the sweat specimen.

Two other methods are currently used for the laboratory diagnosis of CF, both of which were originally devised as screening methods: the ion-selective electrode for chloride (*15*) and the sweat conductivity test (*16*). We will consider only the first method in this report, because it provides a quantitative estimate of the chloride ion in sweat and is in use in some cystic fibrosis study centers.

Materials and Methods

Instrumentation

We used a Flame Photometer, Model 143 (Instrumentation Laboratories, Inc., Watertown, Mass. 02172), a GMT 10 Chloride Titrator (Radiometer, Copenhagen, Denmark), and the Specific Ion Meter, Model 401, with

chloride electrode 97-17 (Orion Research Inc., Cambridge, Mass. 02139).

Reagents

"Ion-To-Gel" (pilocarpine nitrate, 640 mg/liter, in a gel) from TLC Corp., Miami, Fla. 33176, or filter paper pads soaked with pilocarpine solution (640 mg/liter).

"Electr-O-Gel" (potassium sulfate, 10 g/liter, in a gel) from TLC Corp., or filter paper pads soaked with potassium sulfate (10 g/liter).

Methods

Subjects. We studied a total of 1270 subjects. The control group consisted of 595 males and 472 females between the ages of two days to 37 years, including hospital staff members, students, outpatients, and patients admitted to the hospital's pediatric ward. The patients were children with complaints of bronchitis, bronchiectasis, pneumonia, celiac disease, asthma, and complaints with tonsils or adenoids.

The CF group consisted of 104 males and 99 females ranging in age from three months to 21 years.

Pilocarpine iontophoresis (12). The surface of the forearm is washed with de-ionized water and blotted with gauze, Ion-To-Gel is smeared on the flexor surface of the arm, for the positive electrode, and Electro-O-Gel on the extensor surface of the arm, for the negative electrode. Alternatively, as indicated above, filter paper or felt pads soaked with the corresponding reagents may be used. (We find the gel easier to use on small children than soaked filter paper pads because it ensures proper contact between the skin and the electrode; if there is faulty contact, a burn may result and sweat will not be induced.)

The source of electrical current can either be constructed at low cost (*12*) or purchased commercially (Farrall Instrument Co., Grand Island, Neb.). The current is set at 2 to 5 mA for 5 min, and then the electrodes are removed. The area of the positive electrode is left untouched for 5 min before washing with de-ionized water. The area must be dried thoroughly so as not to dilute the sweat, which will be collected on filter paper for quantitative analysis. Alternatively, the ion-selective electrode is touched to the skin for a direct reading by the meter.

Sweat collection on filter paper. Circular Whatman No. 42 papers, 2.5 cm in diameter, are washed three times with distilled water in a beaker, dried in an oven at 100 °C for 20 min, and placed into separate small screw-top plastic vials (with use of forceps). The vials are labeled, weighed, and the weights recorded. After sweat induction and washing and drying of the area of the positive electrode, a piece of the prepared filter paper is removed from its vial and placed on that area. A 7 × 7 cm, square of clear plastic wrap is secured over the filter paper patch, with waterproof tape around the edges. After 30 min the patch is removed with forceps and *immediately* placed into the original vial, sealed, and reweighed. Evaporation is rapid, so this step is critical if falsely high sodium and chloride values are to be avoided. If the weight of the sweat specimen exceeds 50 mg it is diluted with 2 ml of water; if it is less the specimen is discarded and the sweat induction is repeated.

Electrolyte analysis. (1) Ion-selective electrode method: After sweat induction by pilocarpine iontophoresis, chloride on the skin is directly measured with an ion-selective electrode by placing the electrode directly on the skin, according to the manufacturer's instructions.[3]

(2) Modified ion-selective electrode method: The ion-selective electrode was also touched directly to the sweat-containing filter paper and the chloride concentration determined.

(3) Gibson–Cooke Method: Sodium and potassium were measured by flame photometry after a 20-fold dilution of the sweat solution with lithium diluent (15 mmol/liter). Chloride was measured by silver titration with no further dilution. All analyses described above were done in duplicate and standards were used at concentrations of 12, 25, 75, and 100 mmol/liter of each electrolyte.

We made several comparisons to test the reliability of the three methods, as performed by a well-trained operator. The performance of an operator untrained in sweat collection was then compared with that of the operator who had done the sweat collection for a year or more. All comparisons were made by using the coefficient of correlation, r, calculated with the following equation:

$$r = \frac{\Sigma xy - \frac{\Sigma x \Sigma y}{n}}{\sqrt{\left(\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right)\left(\Sigma y^2 - \frac{(\Sigma y)^2}{n}\right)}}$$

In experiment 2 of Table 2, iontophoresis was done on each arm separately, with a 5–10 min interval between arms.

Results

Figure 1 illustrates the distribution of sodium values from 1270 individual first analyses. Results for sodium and chloride below 40 mmol/liter were considered normal and positive if they exceeded 60 mmol/liter. Included in Figure 1 are only those results where the difference between the chloride results and the sodium results did not exceed 30 mmol/liter.[4] The predictive value of the first sweat test was calculated according to Galen and Gambino (*17*) as shown on Table 1; the iontophoresis test is shown to be 84% reliable by these criteria, i.e., the false-positive rate is 16%. Retesting increases the reliability or predictive value to 93%, and decreases the number of false positives accordingly (Table 2). The efficiency and sensitivity are not changed by retesting. Again, we stress that all positive and almost

[3] Instruction Booklet for Orion Instrument No. 417, Skin Chloride Measuring System. Orion Research Inc., Cambridge, Mass. 02139.

[4] Insufficient sweat was collected from 26 individuals and seven tests had to be repeated because Na^+ and Cl^- values differed by >30 mmol/liter.

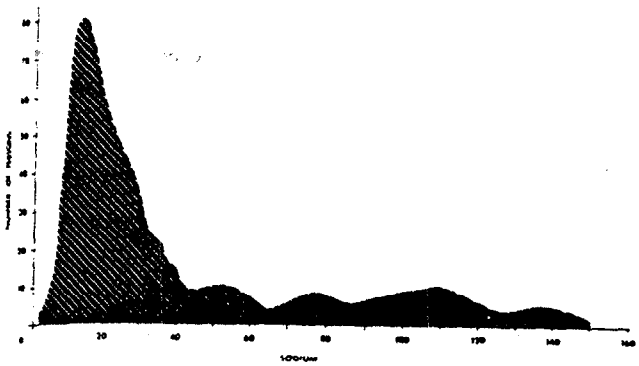

Fig. 1. Frequency distribution of sodium in sweat in 1270 people
Units on abscissa: mmol/l

Table 1. Iontophoresis Sweat Test: First-Test Results

Positive[a] sweat test	Negative[b] sweat test	Totals
Children with cystic fibrosis		
202	1	203
Controls		
38	1029	1067
Totals		
240	30	1270

Predictive value (21)

$$\text{Predictive value of} = \frac{202}{240} = 84\%$$

$$\text{Efficiency} = \frac{202 + 1067}{1270} = 99\%$$

Sensitivity = 202/203 = 99%

Specificity = 1024/1067 = 96%

False-positive rate = 100% − 84% = 16%

[a] Results >40 mmol/liter for controls were called positive
[b] Results <60 mmol/liter for CP patients was considered negative.

Table 2. Final Results of Sweat Test after Retesting

Positive[a] sweat test	Negative[a] sweat test	Totals
Children with cystic fibrosis		
202	1	203
Controls		
16	1051	1067
Totals		
218	1052	1270

Predictive value of

$$\text{a positive result} = \frac{202}{218} = 92\%$$

Specificity = 1051/1067 = 98%

False-positive rate = 100% − 92% = 8%

[a] Results <60 mmol/liter from one to three sweat tests were considered normal, those >60 mmol/liter from one to three sweat tests were considered positive

Table 3. Comparison of Electrolyte Measurement by Different Methods

Experiment no.	Methods[a]	No. subjects	Comparison	Correlation coeff., r
1	a and b	57	Cl^- vs. Cl^-	.57
2	c and b	49	Cl^- vs. Cl^-	.68
3	b	1270	Na^+ vs. Cl^-	.97

[a] a, ion-selective electrode, directly on skin; b, silver titration and flame photometry of Cl^- and Na^+; c, ion-selective electrode used on filter paper specimen.

Table 4. Correlation of Results of Various Measurements of Na^+, Cl^-, and K^+

Experiment no.	Methods	No. subjects[a]	Comparison	r
1	Repeat test done on different days by same technician[b]	234	Cl^- vs. Cl^-	.94
			Na^+ vs. Na^+	.91
2	Test done simultaneously on each arm by same technician[c]	167	Cl^- vs. Cl^-	.95
			Na^+ vs. Na^+	.93
3	Test done by trained technician and by untrained technician on same day	54	Cl^- vs. Cl^-	.81
			Na^+ vs. Na^+	.83
4	Flame-photometry and chloride titration	192	Na^+ vs. K^+	.20
			Cl^- vs. K^+	.13

[a] Subjects included patients with cystic fibrosis. The sweat sodium ranged from 3–145 mmol/liter.
[b] Data are only for tests with sodium and chloride values under 50 mmol/liter on the first test.
[c] Data are for tests with sodium and chloride values from 9–132 mmol/liter.

positive results of this test (over 40 mmol/liter) must be repeated at least once.

The correlation between results of the various analyses is shown in Table 3. The results with the ion-selective electrode do not correlate well with those from the Gibson–Cooke method, as seen in experiment 1. Indeed, we found that the reading on the meter could be changed by simply changing the pressure of the electrode on the arm or by varying the angle between the electrode and the arm surface. These variables were easier to control when applying the electrode to filter paper on a flat surface. The accuracy of the Gibson–Cooke test was also better, because the sweat on the filter paper is weighed. As stated, if less than 50 mg of sweat was collected, the test was repeated. In contrast, it is impossible to estimate the amount of sweat when

the electrode is directly applied to the arm. The correlation of various measurements of Na^+, Cl^-, and K^+ are shown on Table 4. The comparisons show that a trained operator will get reproducible results whether tests are done on different arms on the same day or on different days. Results from untrained technicians do not correlate well with results achieved by experienced personnel. The K^+ values do not correlate at all with Cl^- or Na^+ results.

Discussion

A recent survey of 86 hospitals in one state showed that 41% offered no sweat test and only 6% used the Gibson–Cooke technique (*14*). An ion-selective electrode was used by 30% of the hospitals as the only diagnostic test for CF. There have been reports that this procedure is a satisfactory test (*5, 6*) but the manufacturer stresses that the instrument is to be used as a screening test only (*18*) and a recent report by the National Academy of Sciences (*15*) suggests that the meticulous attention to detail that is required[5,6] precludes use of this method in community hospital laboratories. A survey in the same report, covering 145 Cystic Fibrosis Centers around the country, revealed that only 62% used the Gibson–Cooke technique while 19% used the ion-selective electrode exclusively.

When the sweat test was first described in its present form 16 years ago, there were warnings that the test should only be done in certain specialized laboratories (*12, 13*). This is understandable, because the test was new and specimen collection and analysis was technically difficult. Many more laboratories should now be able to offer the test with validity after the appropriate control studies are done and standardization is assured.

The data shown in Table 3 suggest that the quantitative analysis of sodium and chloride by an experienced operator is the method of choice for the laboratory diagnosis of CF. In a situation where chloride titration is not readily available, the ion-selective electrode used on weighed filter paper can give satisfactory results for chloride, but this analysis should always be done twice and always checked by flame-photometric sodium analysis.

We conclude that a good Na^+/Cl^- sweat test for CF should meet the following criteria: (*a*) both sodium and chloride are determined, in duplicate; (*b*) the averaged results are within 30 mmol/liter of each other; (*c*) 50 mg or more of sweat is analyzed; (*d*) the test is repeated if values for either sodium or chloride exceed 40 mmol/liter; (*e*) the report to the physician must include the weight of the sweat specimen and the values for sodium and chloride; and (*f*) only the same trained operator should collect all specimens.

We thank Laura Martinez and Marilyn Alicea for their expert technical assistance. This work was supported in part by Cystic Fibrosis Research Grant Y-5263 and HEW, Bureau of Community Health Services, MCHS, Project No. 288.

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STUDIES IN CYSTIC FIBROSIS: DETERMINATION OF SWEAT ELECTROLYTES IN SITU WITH DIRECT READING ELECTRODES

BY

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STUDIES IN CYSTIC FIBROSIS: DETERMINATION OF SWEAT ELECTROLYTES IN SITU WITH DIRECT READING ELECTRODES

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From the Division of Clinical Laboratories and the Department of Medicine, Children's Hospital Medical Center, the Department of Pediatrics, Harvard Medical School, and the Children's Cancer Research Foundation

ABSTRACT. An evaluation is made of newly developed specific-ion electrodes for determining sodium and chloride on the skin surface following the stimulation of the sweat glands by pilocarpine iontophoresis.

This procedure offers a rapid (5 to 6 minutes), convenient, and safe means for large-scale cystic fibrosis screening programs for screening infants and children.

Caution is urged in the evaluation of the reading since a failure to sweat may result in a misleading value. *Pediatrics*, 43:794, 1969, CYSTIC FIBROSIS, SWEAT ELECTROLYTES, SODIUM ELECTRODE, CHLORIDE ELECTRODE.

THE STIMULATION of sweat by pilocarpine iontophoresis[1,2] followed by a chemical analysis of sodium and chloride[3] is currently the preferred method for confirming the diagnosis of cystic fibrosis (CF). This procedure is not practical for screening large groups since the test requires about 45 minutes.

The first survey for detecting children with CF in a school population was conducted in 1958 using the chloride imprint or "plate test."[4] This semi-quantitative procedure is still used by us for screening patients in a hospital setting. More recent screening methods utilizing quantitative determinations of electrolyte activity directly on the skin were described by Goldbloom and Sekelj,[5] who measured sodium, and Warwick and Hansen,[6] who measured chloride after local thermal stimulation of sweat.

The present report summarizes our experience with electrode measurements of Na^+ and Cl^- on the skin with and without local stimulation of sweat by either heat or pilocarpine iontophoresis.

PROCEDURES AND RESULTS

The Goldbloom and Sekelj[5] procedure consisted of placing a glass-sodium* electrode directly on a washed skin area and calculating sodium activity from the developed electrical potential obtained by an expanded-scale pH meter.

They tested 24 patients with CF and 36 controls without the use of artificial sweat stimulation. We used similar instrumentation and methodology on 27 patients with CF and 28 controls. The comparative data is presented in Table I.

Although good agreement is observed in the mean sodium activities of patients with CF, there is an overlap between the two groups. We also failed to identify three known patients with CF who gave readings of 20, 25, and 50 mEq Na^+ per liter of sweat. The first of these patients was dehydrated and had scaly, dry skin. We would have disqualified this patient for testing with the skin electrode by observation. The other two patients showed no visible indication of failure to sweat. Two controls gave readings of 70 and 80 mEq Na^+ per liter.

Warwick and Hansen[6] and Hansen, *et al.*[7] introduced two important changes in the

* Sodium combination electrode No. 31142, Beckman Instruments, Fullerton, California.

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foregoing procedure. They measured chloride rather than sodium activity, making use of a newly developed, solid-state chloride electrode, and they stimulated local sweating by placing small heated aluminum cylinders or "gel packs"† over pliofilm covered skin to prevent sweat evaporation, contaminations, and burns. As part of a comprehensive study involving 1,156 subjects, they evaluated three different electrodes and four meters supplied by Beckman Instruments,‡ Radiometer Corporation,‖ Orion Research, Inc.¶ and United Systems Company.#

We repeated Warwick's and Hansen's studies using a more recent Orion chloride electrode and a direct reading meter calibrated in mEq Cl/liter.** We also stimulated sweating by placing heated "gel-packs" over a washed area of skin and obtained higher but similar values to those observed previously. Our comparative results are presented in Table I. We standardized the electrode in aqueous solutions of known Cl^- concentration at room temperature instead of at 37°C as was done by Warwick and Hansen.[6] We did not find it necessary to correct for temperature differences between the skin and electrode.

Our preferred procedure was based on sweat stimulation by pilocarpine iontophoresis followed by an immediate determination of Cl^- activity on the skin by a chloride electrode as follows:

1. The Cl^- electrode is filled with an internal filling solution of (1M KNO_3, 0.030M KCl, and saturated AgCl) supplied by the manufacturer.

2. The meter is calibrated with standard solutions of 20 and 100 mEq Cl^- per liter. These values approximate the mean normal and mean CF Cl^- levels in sweat. Readings obtained between these two values are reproducible to within ±2 mEq Cl^-/liter.

3. After calibration, the electrode may be dipped in ion-free water or it may be placed in a vertical position with the sensing crystal resting on several gauze pads moistened with distilled water. In the latter position the electrode is ready for immediate application.

4. A 64 mg/100 ml pilocarpine-HCl soaked felt pad is placed in the large slot and an 0.01 M H_2SO_4 soaked pad is placed in the small slot of the iontophoresis attachment (Fig. 1). The entire assembly is strapped on the flexor surface of the forearm for 5 minutes, during which time sweat stimulation is accomplished with a current of 1 milliampere. (In testing newborns or infants the iontophoresis assembly may be held on the back.)

5. The iontophoresis attachment is removed and the stimulated area is washed with a gauze pad moistened with ion-free water.

6. The skin surface and electrode are blotted dry (with gauze) and the electrode is quickly placed on the sweating area (Fig. 2).

7. The meter pointer should stabilize

† Gel pack system. Chattanooga Pharmacal Corporation, Chattanooga, Tennessee.

‡ No. 78727 V. Electrode; No. 76 Expanded scale pH meter, Beckman Instruments, Fullerton, California.

‖ Type PKS 67011 electrode Model 27 pH meter, Radiometer Corporation, Copenhagen, Denmark.

¶ Model 94-17 electrode, Model 401 specific-ion meter, Orion Research Inc., 11 Blackstone Street, Cambridge, Massachusetts.

Model 402 meter, United Systems Company, Dayton, Ohio.

** Orion Model 96-17 electrode and series 400 specific-ion meter, 11 Blackstone Street, Cambridge, Massachusetts.

FIG. 1. Iontophoresis attachment showing pilocarpine-impregnated pad (right) and H_2SO_4-impregnated pad (left).

TABLE I

DIRECT SKIN ELECTROLYTE DETERMINATION WITH ION-SPECIFIC ELECTRODES

Data	*Sodium mEq/l*		*Chloride mEq/l*	
	Spontaneous Sweating		*Sweating Induced by Heat*	
	Goldbloom and Sekelj[3]	*Our Values*	*Warwick and Hansen*[6]	*Our Values*
Number of patients with CF	26	27	49	21
Mean	131.1	135	89.2	109
Range	54–200	20–360	55–130	40–150
S.D.	42.5	54	18.6	35
Number of controls	36	28	28	30
Mean	26.8	36	13	21
Range	17–40	10–80	5–30	10–60
S.D.	5.2	9.6	6.5	9.7

after 10 to 20 seconds and a reading is taken directly in milliequivalents Cl^- per liter.

Since sweating continues for about 5 minutes, additional readings may be taken. However, steps (5) and (6) should be repeated prior to each application of the electrode to the surface of the skin. Recalibration of the electrode is advisable after several determinations.

The results are presented in Table II and Figure 3. All but six observations are clearly defined as typical of CF or non CF. The six subjects with borderline values also had similar chloride levels by standard techniques.

DISCUSSION AND CONCLUSION

The direct-reading skin Cl^- electrode is an unusually sensitive device, having a limit of detection of about 0.05 mEq Cl^-/liter. The reproducibility of the electrode in aqueous solution is about $\pm 1\%$, within a range 10 to 150 mEq/l. When applied to the skin, the "ideal" conditions which prevail in aqueous solutions do not hold. We find that the primary source of error is due to the rapid evaporation of sweat from the stimulated area. To minimize this error, the electrode is placed on the skin immediately following the wiping of the skin with a gauze pad (step 6 in the procedure). Erroneously high initial readings tend to drift downward if the electrode is held firmly in place while the patient continues to sweat. Excessive washing or moistening of the skin after sweat stimulation (step 5 in the procedure) will result in erroneously low readings. Both types of error can be overcome by practice. The newer, "solid-state" electrodes were found to be insensitive to the pressure with which they were applied to the skin. This was one of the major drawbacks of the earlier sodium and chloride electrodes which were "pressure-sensitive."

Since some subjects fail to sweat sufficiently, we feel that artificial sweat stimulation, either thermal or electrochemical, is essential. A few subjects may fail to sweat sufficiently, even after sweat stimulation;

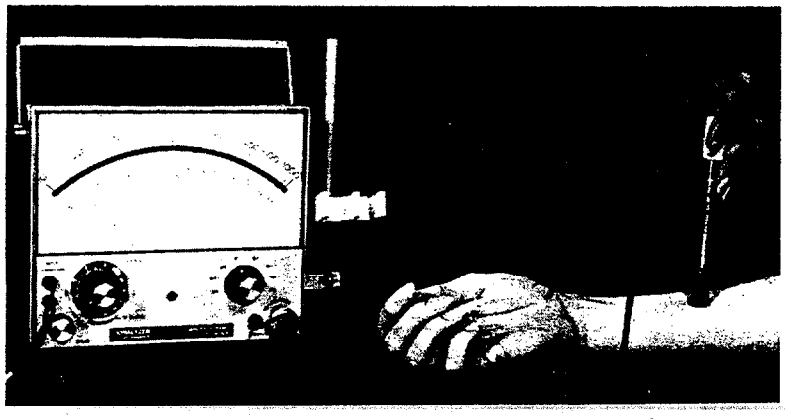

FIG. 2. The electrode in position during sweat chloride activity determination (meter—upper scale calibrated in mEq Cl^-/liter).

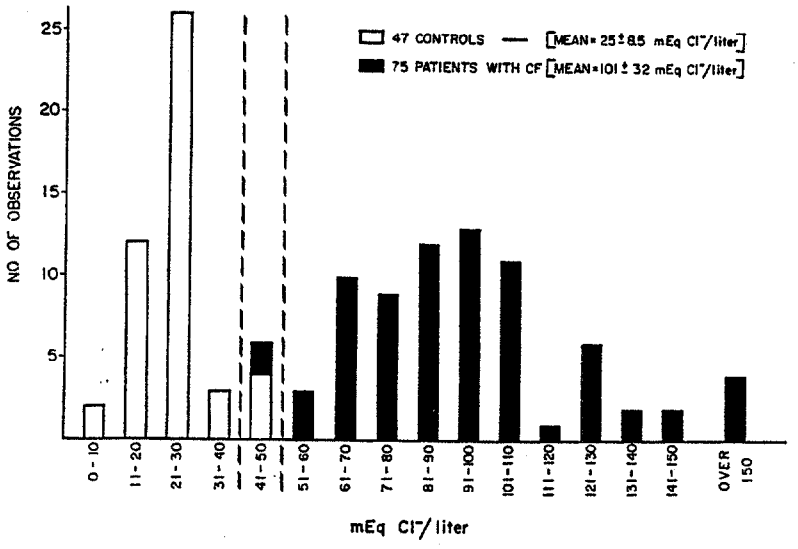

FIG. 3. Sweat chloride determined with an electrode (following stimulation of sweat by pilocarpine iontophoresis).

these individuals may be misdiagnosed. When borderline values (i.e., between 40 and 60 mEq/l) are obtained, we find similar values by other techniques which make interpretation extremely difficult. Only careful clinical and repeated laboratory observations can eventually properly identify these patients. We agree with Warwick and Hansen[6] that the thermal stimulation of sweat and subsequent direct chloride measurement is indeed a promising procedure for both diagnosis and large scale screening of newborns, infants, and children. However, we disagree on a point of procedure. Compensatory corrections for differences between skin and room temperatures are not required as the temperature of the crystal sensor of the electrode does not increase appreciably during the 10 to 20 seconds required to obtain a stable reading of Cl^- activity on the skin. This electrode has considerable mass compared to the small surface area that comes in contact with the skin. Heat from the warmer skin to the cooler electrode is conducted only through this surface area, while the remainder of the electrode, including about 5 to 10 ml of filling solution, in direct contact with the inner face of the sensing element remains at room temperature. Conditions of temperature equilibrium between the skin and the Cl^- sensing element are not established; consequently, a correction to compensate for possible temperature differential of about 15 or 17°C is not warranted. Warwick and Hansen[6] compensated for a temperature differential of about 17°C, and by doing so obtained lower mean values for both the controls and patients with CF. This did not detract from the quality of

TABLE II

COMPARISON OF DIRECT SKIN CHLORIDE IN MEQ/L BY ELECTRODE AND BY PILOCARPINE IONTOPHORESIS AND CHEMICAL ANALYSIS[3]

Data	*By Electrode Following Pilocarpine Iontophoresis*	*By Chemistry[3] Following Pilocarpine Iontophoresis*
Number of patients with CF	75	68
Mean	101	116
Range	45–200	52–148
S.D.	32	18
Number of controls	47	34
Mean	25	23
Range	10–50	12–39
S.D.	8.5	6.8

their results as the separation and the ratios between the group mean values remained constant.

Sweat stimulation by pilocarpine iontophoresis may be more convenient than the thermal method as no preparatory steps are required and the equipment is ready for immediate use. The danger from skin burns is eliminated through the design of the current-limited battery power source which keeps the current for iontophoresis at less than 1.5 milliamperes. Additional readings may be obtained for as long as sweating continues. These repeat measurements are done on newly formed sweat and are independent of the initial determination.

In our experience with over 10,000 pilocarpine iontophoresis sweat tests, adequate sweat was produced for analysis in nearly every case.[8] The exceptions were few and included the rare patients with ectodermal dysplasia, scleroderma, hypothyroidism, some severely dehydrated and malnourished infants, and some premature infants. Less sweat is needed for in situ chloride measurements than for conventional sweat tests.

The measurements in this report were made with a current limited to 1 milliampere. When the current is increased to 1.5 milliampere, a smaller number of failures or "non-sweaters" were noted. The application of the iodine-starch test immediately after completing the reading may be used to indicate whether local sweating did occur.

At present we employ our routine quantitative pilocarpine iontophoresis sweat test in confirming the diagnosis of CF and in studying patients with borderline sweat tests.

SUMMARY

Three proposed procedures for the in situ determination of sweat chlorides are evaluated. An adequate amount of sweat must be available on the skin surface; hence, stimulation of sweating is essential. We prefer sweat stimulation by local pilocarpine iontophoresis rather than by local heat. This method is convenient, safe, and reliable, and it can be applied to large-scale screening programs in newborns, infants, and children. The use of direct-reading skin chloride electrodes have the following advantages over the conventional sweat test.

1. The time required for the entire procedure is 5 to 6 minutes instead of 45 minutes. At least two additional and independent determinations may be made within 1 or 2 minutes.

2. The results are obtained directly in mEq Cl^-/liter of sweat. No additional quantitative determinations are necessary, and no computations are involved.

3. Less skill and knowledge of analytical chemistry are required of the operator. The technique can be acquired in a relatively short time.

4. The equipment, including the sweat stimulating attachment, is compact, portable (battery operated), light (5 lb), and ready for immediate use.

It is important to recognize that misleading information will result if the subject fails to sweat.

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A TEST FOR CONCENTRATION OF ELECTROLYTES IN SWEAT IN CYSTIC FIBROSIS OF THE PANCREAS UTILIZING PILOCARPINE BY IONTOPHORESIS

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THE DISCOVERY OF Darling and co-workers[1,2] that there is a high concentration of sodium and chloride in the sweat of patients with cystic fibrosis of the pancreas has been widely applied in the diagnosis of the disease. When these authors tested 43 patients with this disease, they found that all of the patients had concentrations of chloride in sweat above 60 meq/l, with a mean value of 106 meq/l; 50 controls all had values below 80 meq/l and only 3 were above 60 meq/l. In many clinics the sweat test is performed by placing the patient's body in a plastic bag to stimulate sweating as suggested by Shwachman *et al.*[3]

Although the plastic-bag method is both simple and reliable, there are certain difficulties with the procedure: 1) The time required to obtain a sufficient quantity of sweat is variable. 2) Some infants become hyperpyrexic after remaining in the bag only 30 minutes. 3) Other patients, especially adults, require 2 hours. 4) The procedure is also uncomfortable, and younger patients often require constant attention to prevent a forceful exit from the bag. Some clinicians have put hot-water bottles into the bag with the patient when the bag alone fails to produce sufficient sweating. This procedure is not to be recommended since Misch and Holden[4] have reported a fatal heat stroke following this practice.

An alternate method suggested by Mauer and West[5] consists of injection of a cholinergic drug (furtrethonium iodide or bethanechol) intradermally and collecting sweat over the point of injection. The injection is obviously painful. The oral administration of cholinergic drugs has not been recommended as a diaphoretic stimulus for this test; such a procedure would be dangerous because of systemic effects.

The method to be described obviates these difficulties by avoiding both the danger of heat stress and the pain of injection. Pilocarpine is introduced into the skin by iontophoresis where it produces localized sweating. The process of iontophoresis is almost completely painless and requires only 5 minutes. Rapid sweating continues for about 30 minutes after stimulation, during which time an adequate collection can be made.

PATIENT MATERIAL

Twenty-five patients with cystic fibrosis of the pancreas were tested. In all of these children there was a history of excessive appetite, bulky foul stools and recurrent pulmonary disease. All patients' physical findings and roentgenograms of the lungs were suggestive of the disease. Thirteen patients had duodenal aspirations showing absent or greatly diminished proteolytic activity. Eleven of these patients had sweat collected by the bag method and were found to have concentrations of chloride in the sweat ranging from 107 to 128 meq/l.

Seventeen asymptomatic relatives of these patients were tested.

Forty-seven individuals not thought to have cystic fibrosis were tested. They were believed to have the following conditions: 20 had asthma; 8 had recurrent bronchitis or tonsillar and adenoidal disease; 3 had hepatitis; 2 had congenital heart disease, each had agammaglobulinemia, nontropical sprue, osteomyelitis, pyelonephritis, pneumonia, bronchiectasis,

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celiac disease, mental retardation, duodenal ulcer, Fanconi's syndrome (renal), and hypertension. Three individuals tested were apparently healthy. None of these patients had both digestive and respiratory disease. Two of them had previously been found to have normal concentrations of chloride in sweat after a plastic bag had been used to induce sweating.

EQUIPMENT

Source of Electrical Current

Any source of direct current may be used provided a rheostat is used which allows the application of from 0 to 20 volts across the electrodes and a milliampere-meter is employed on which 2 ma may be accurately read. In the apparatus used in the present study the power is supplied by three 7.5-volt multiple-contact radio batteries. The rheostat has a maximum resistance of 2,500 ohms. The milliampere-meter reads 15 ma full scale and has an interval resistance of 485 ohms. The wiring is shown in Figure 1. Parts cost a total of about $7.00.

Electrodes

Two modified electrocardiograph electrodes are used. The negative one is gauze-covered, while the positive one has a rubber-covered base from which protrudes a circular metal platform 2 cm in diameter and 0.3 cm in height.

Other Equipment

A rubber strap to secure the electrodes; bottles of distilled water; 0.07 normal sodium bicarbonate and 0.2% pilocarpine nitrate (refrigerated when not in use); low-ash filter-paper disks (Schleicher and Schuell, No. 589, Green ribbon), 2.5 cm in diameter; squares of plastic sheet 3 by 3 cm; forceps; waterproof adhesive tape, ½ inch width; an analytic balance; weighing bottles; and material for chloride analysis (see below).

PROCEDURE

First the electrodes are prepared. An unweighed disk of filter paper is placed upon the

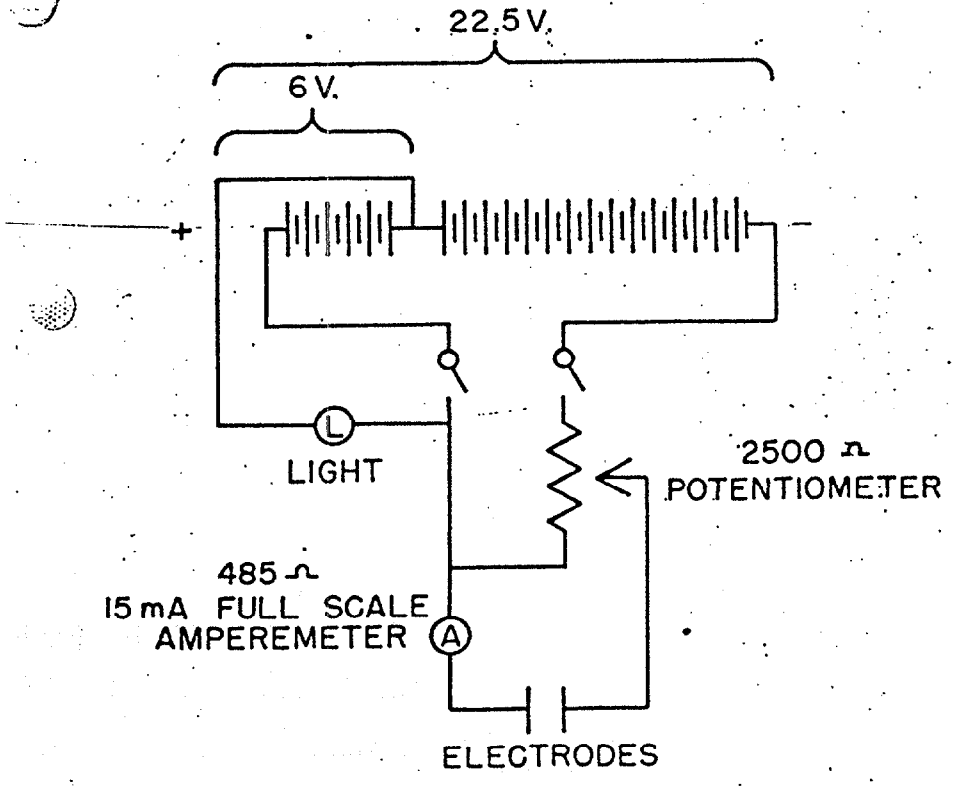

FIG. 1. Wiring diagram of source of electric current. (For description see text.)

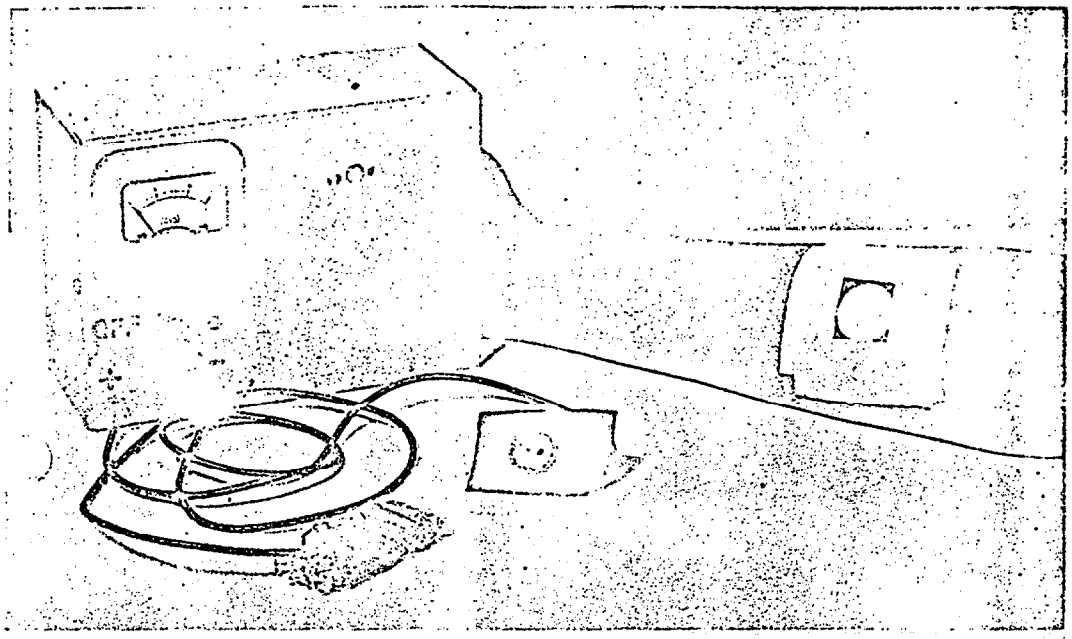

FIG. 2. Apparatus for delivering electric current; the electrodes; and the collecting system applied to a forearm.

ed circular platform on the positive electde. Four drops (about 0.2 ml) of the pilocarpine solution are placed on the filter paper. Then a few milliliters of the solution of sodium bicarbonate are poured on the gauze-covered negative electrode. The positive electrode is applied to the flexor surface of the forearm and the negative one is placed behind it on the extensor surface. The rubber strap is used to secure the electrodes. The pilocarpine-saturated filter paper should completely cover the exposed metal on the positive electrode. Direct contact between metal and skin reduces iontophoresis and may cause a small burn.

When in place, the electrodes are connected to the power source. Slowly, over a 5- to 10-second period, the current is raised to 2 ma. Iontophoresis, thus begun, is continued for 5 minutes. Occasionally slight discomfort is noticed during the process. This is caused by poor contact between skin and electrode and may be easily alleviated by pressing momentarily on the offending electrode or by tightening the rubber strap.

After iontophoresis is complete, the electrodes are removed and the skin is cleansed with distilled water and dried with gauze. Then a disk of filter paper, similar to that just used on the positive electrode, is removed from a previously weighed bottle with forceps and placed over the area that was iontophoresed. The paper is covered with a plastic square, the edges of which are each carefully secured with four strips of adhesive tape.

After a collection period of 30 minutes, the plastic square is removed and the now moist filter paper quickly returned to the weighed bottle and reweighed. Figure 2 shows the apparatus.

CHLORIDE DETERMINATION

Polarographic Method

In these collections between 18 and 135 mg of sweat were obtained in each test. The average rates of sweating were, therefore, 1.2 to 9.2 gm/m²/min and are comparable to those obtained using a bag. However, the absolute quantity of sweat collected is small and a fairly sensitive method of chloride analysis must be used. For the determinations in this study the polarographic method of Zimmerman and Layton[6] was employed. The method was modified by using 0.1 N sulfuric acid for the supporting electrolyte, as suggested by Schönholzer,[7] instead of phosphotungstic acid; 4 ml of the 0.1 N sulfuric acid was used to dilute the samples.

Other Methods

Laboratories that do not have a polarograph could still use iontophoretic stimulation, either by using larger electrodes (see below) or by using some other micro-method of analysis. The chloride content of the specimen could be determined by the method of Cotlove *et al.*,[8] or the sodium content could be determined by flame photometry.

ALTERNATIVE PROCEDURE

The procedure described represents our final adaptation. However, as will be mentioned later, some of the tests were done using an alternate method in which 3 by 3 inch squares of gauze were used for iontophoresis and collection instead of the filter-paper disks. In this case 4 ml of a 0.05% or 0.10% pilocarpine nitrate solution was iontophoresed with a current of 4 ma for 15 minutes. Suitably larger electrodes were used. The collection periods varied from 15 to 90 minutes (the prolonged collections were found to add little sweat). This procedure is quite satisfactory, but somewhat less convenient. It has one advantage in that larger quantities of sweat are collected than when using the filter-paper disks. The chloride determinations were performed by the method of Schales and Schales.[9]

RESULTS

Using the procedure employing the 3 by 3 inch squares (Alternative Procedure), 34 tests were performed. An average of 692 mg of sweat was obtained in each collection, with a range of 150 to 1,620 mg. The chloride concentrations found are tabulated in Table I.

Using the method employing 2.5 cm diameter disks of filter paper, 55 tests were performed. An average of 76 mg of sweat was obtained in each collection, with a range of 18 to 135 mg. In only four collections were less than 40 mg obtained. The chloride concentrations found are tabulated in Table II.

TABLE I

RESULTS OF ANALYSIS OF SWEAT COLLECTED ON 3 BY 3 INCH GAUZE SQUARES (ALTERNATE PROCEDURE)

	No.	Chloride in Sweat (meq/l)	
		Average	Range
Patients with cystic fibrosis	14	110.1	84–136
Relatives of patients	12	33.9	12– 57
Controls	8	45.4	11– 60

TABLE II

RESULTS OF ANALYSIS OF SWEAT COLLECTED ON 2.5 CM DIAMETER FILTER-PAPER DISKS

	No.	Chloride in Sweat (meq/l)	
		Average	Range
Patients with cystic fibrosis	11	94.8	80–122
Relatives of patients	5	32.5	16– 47
Controls	39	21.1	7– 49

DISCUSSION

The studies indicate that sweat induced by iontophoresis of pilocarpine nitrate is similar to that resulting from elevation of environmental temperature and that the iontophoretic method also gives good diagnostic evidence for or against cystic fibrosis of the pancreas. Twenty-five patients with this disease were tested and all of them were found to have concentrations of chloride in sweat of 80 or more meq/l. None of the controls had a concentration of more than 60 meq/l. Although none of the 17 relatives of the patients had concentrations of chloride in sweat above 57 meq/l, the work of di Sant'Agnese *et al.*[10] would indicate that a larger group of relatives of patients with cystic fibrosis might include individuals with abnormal concentrations.

The test described has appeared to be quite safe. The exact dose of pilocarpine delivered to the patient is impossible to ascertain accurately, because it is quite unlikely that the maximum possible dose, i.e., the quantity of drug placed on the electrode, was actually delivered to the patient. The maximum possible dose was 0.4 mg in the filter-paper method and was 2 to 4 mg when gauze was used. The average oral or hypodermic dose of pilocarpine nitrate is 5 to 10 mg;[11] the fatal dose is unknown, but

100 mg is thought to be dangerous.[11] No systemic effects were observed in any of these tests, although the 4 mg doses were occasionally used on small infants. However, it is to be noted that only the 0.4 mg dose was given to patients with asthma. As this disease might be expected to increase the susceptibility to toxic reactions, the absence of such reactions during 20 tests was reassuring.

The time of iontophoresis is somewhat arbitrary. If the method were completely efficient, the 0.4 mg dose could be delivered by a current of 2 ma in 1¼ minutes instead of the 5 minutes actually used. The choice of the electrolyte on the negative electrode is also quite arbitrary; 0.9% sodium chloride solution or any well-ionized, harmless substance is satisfactory.

The test is particularly well adapted to testing fairly large numbers of patients, consecutively. Most of the individuals with asthma were tested during two afternoons in allergy clinic. Because the collection methods did not require supervision, the time required was about 10 minutes per test.

SUMMARY

A method of performing the test for chloride or sodium in the sweat of patients with cystic fibrosis of the pancreas is presented. It utilizes pilocarpine by iontophoresis and has the advantage of being both rapid and painless. Data are presented to show that the method is reliable.

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